Claims

1. An immunoassay solid support comprising at least one hepatitis C virus (HCV) anti-core antibody and at least one isolated HCV NS3/4a epitope bound thereto.

2. The immunoassay solid support of claim 1, comprising at least two HCV anti-core antibodies bound thereto.

3. The immunoassay solid support of claim 1, wherein said at least one anti-core antibody is directed against an N-terminal region of the HCV core antigen.

4. The immunoassay solid support of claim 3, wherein said at least one anti-core antibody is directed against amino acids 10-53 of HCV, numbered relative to the HCV1 polyprotein sequence.

5. The immunoassay solid support of claim 1, wherein said at least one anti-core antibody is a monoclonal antibody.

6. The immunoassay solid support of claim 1, wherein said NS3/4a epitope is a conformational epitope and comprises the amino acid sequence depicted in Figures 4A-4D.

7. The immunoassay solid support of claim 1, further comprising a multiple epitope fusion antigen bound thereto.

8. The immunoassay solid support of claim 7, wherein said multiple epitope fusion antigen comprises the amino acid sequence depicted in Figures 7A-7F.

9. An immunoassay solid support comprising two hepatitis C virus (HCV) anti-core monoclonal antibodies and an HCV NS3/4a conformational epitope comprising the

amino acid sequence depicted in Figures 4A-4D, bound thereto.

10. The immunoassay solid support of claim 9, wherein said two anti-core antibodies are directed against an N-terminal region of the HCV core antigen.

11. The immunoassay solid support of claim 10, wherein said two anti-core antibodies are directed against amino acids 10-53 of HCV, numbered relative to the HCV1 polyprotein sequence.

12. An immunoassay solid support comprising two hepatitis C virus (HCV) anti-core monoclonal antibodies, an HCV NS3/4a conformational epitope comprising the amino acid sequence depicted in Figures 4A-4D, and a multiple epitope fusion antigen comprising the amino acid sequence depicted in Figures 7A-7F, bound thereto.

13. A method of detecting hepatitis C virus (HCV) infection in a biological sample, said method comprising:

(a) providing an immunoassay solid support according to claim 1;

(b) combining a biological sample with said solid support under conditions which allow HCV antigens and antibodies, when present in the biological sample, to bind to said at least one anti-core antibody and said NS3/4a epitope, respectively;

(c) adding to the solid support from step (b) under complex forming conditions (i) a first detectably labeled antibody, wherein said first detectably labeled antibody is a detectably labeled HCV anti-core antibody, wherein said labeled anti-core antibody is directed against a different HCV core epitope than the at least one anti-core antibody bound to the solid support; (ii) an antigen that reacts with an HCV antibody from the biological sample reactive with said NS3/4a epitope; and (iii) a second detectably labeled antibody, wherein said second detectably labeled antibody is reactive with the antigen of (ii);

(d) detecting complexes formed between the antibodies and antigens, if any, as an indication of HCV infection in the biological sample.

14. The method of claim 13, wherein said at least one anti-core antibody is directed against an N-terminal region of the HCV core antigen and said detectably labeled HCV anti-core antibody is directed against a C-terminal region of the HCV core antigen.

15. The method of claim 14, wherein said at least one anti-core antibody is directed against amino acids 10-53 of HCV, numbered relative to the HCV1 polyprotein sequence and said detectably labeled HCV anti-core antibody is directed against amino acids 120-130 of HCV, numbered relative to the HCV1 polyprotein sequence.

16. The method of claim 13, wherein said antigen that reacts with an HCV antibody from the biological sample comprises an epitope from the c33c region of the HCV polyprotein.

17. The method of claim 16, wherein the c33c epitope is fused with a human superoxide dismutase (hSOD) amino acid sequence and the second detectably labeled antibody is reactive with said hSOD amino acid sequence.

18. The method of claim 13, wherein said NS3/4a epitope is a conformational epitope and comprises the amino acid sequence depicted in Figures 4A-4D.

19. A method of detecting hepatitis C virus (HCV) infection in a biological sample, said method comprising:

(a) providing an immunoassay solid support according to claim 2;

(b) combining a biological sample with said solid support under conditions which allow HCV antigens and antibodies, when present in the biological sample, to bind to the said at least two anti-core antibodies and said NS3/4a epitope, respectively;

(c) adding to the solid support from step (b) under complex forming conditions (i) a first detectably labeled antibody, wherein said first detectably labeled antibody is a detectably labeled HCV anti-core antibody, wherein said labeled anti-core antibody is

directed against a different HCV core epitope than the at least two anti-core antibodies bound to the solid support; (ii) an epitope from the c33c region of the HCV polyprotein fused to an hSOD amino acid sequence; and (iii) a second detectably labeled antibody, wherein said second detectably labeled antibody is reactive with said hSOD amino acid sequence;

(d) detecting complexes formed between the antibodies and antigens, if any, as an indication of HCV infection in the biological sample.

20. The method of claim 19, wherein said NS3/4a epitope is a conformational epitope and comprises the amino acid sequence depicted in Figures 4A-4D.

21. A method of detecting hepatitis C virus (HCV) infection in a biological sample, said method comprising:

(a) providing an immunoassay solid support according to claim 9;

(b) combining a biological sample with said solid support under conditions which allow HCV antigens and antibodies, when present in the biological sample, to bind to the said at least two anti-core antibodies and said NS3/4a conformational epitope, respectively;

(c) adding to the solid support from step (b) under complex forming conditions (i) a first detectably labeled antibody, wherein said first detectably labeled antibody is a detectably labeled HCV anti-core antibody, wherein said labeled anti-core antibody is directed against a different HCV core epitope than the at least two anti-core antibodies bound to the solid support; (ii) an epitope from the c33c region of the HCV polyprotein fused to an hSOD amino acid sequence; and (iii) a second detectably labeled antibody, wherein said second detectably labeled antibody is reactive with said hSOD amino acid sequence;

(d) detecting complexes formed between the antibodies and antigens, if any, as an indication of HCV infection in the biological sample.

22. The method of claim 21, wherein said at least two anti-core antibodies are

directed against an N-terminal region of the HCV core antigen and said detectably labeled HCV anti-core antibody is directed against a C-terminal region of the HCV core antigen.

23. The method of claim 22, wherein said at least two anti-core antibodies are directed against amino acids 10-53 of HCV, numbered relative to the HCV1 polyprotein sequence and said detectably labeled HCV anti-core antibody is directed against amino acids 120-130 of HCV, numbered relative to the HCV1 polyprotein sequence.

24. A method of detecting hepatitis C virus (HCV) infection in a biological sample, said method comprising:

(a) providing an immunoassay solid support according to claim 7;

(b) combining a biological sample with said solid support under conditions which allow HCV antigens and antibodies, when present in the biological sample, to bind to said at least one anti-core antibody, said NS3/4a epitope, and said multiple epitope fusion antigen;

(c) adding to the solid support from step (b) under complex forming conditions (i) a first detectably labeled antibody, wherein said first detectably labeled antibody is a detectably labeled HCV anti-core antibody, wherein said labeled anti-core antibody is directed against a different HCV core epitope than the at least one anti-core antibody bound to the solid support; (ii) first and second antigens that react with an HCV antibody from the biological sample reactive with said NS3/4a epitope and said multiple epitope fusion antigen, respectively; and (iii) a second detectably labeled antibody, wherein said second detectably labeled antibody is reactive with the antigens of (ii);

(d) detecting complexes formed between the antibodies and antigens, if any, as an indication of HCV infection in the biological sample.

25. The method of claim 24, wherein said at least one anti-core antibody is directed against an N-terminal region of the HCV core antigen and said first detectably labeled HCV anti-core antibody is directed against a C-terminal region of the HCV core

antigen.

26. The method of claim 25, wherein said at least one anti-core antibody is directed against amino acids 10-53 of HCV, numbered relative to the HCV1 polyprotein sequence and said detectably labeled HCV anti-core antibody is directed against amino acids 120-130 of HCV, numbered relative to the HCV1 polyprotein sequence.

27. The method of claim 24, wherein said first antigen that reacts with an HCV antibody from the biological sample comprises an epitope from the c33c region of the HCV polyprotein.

28. The method of claim 27, wherein the c33c epitope is fused with a human superoxide dismutase (hSOD) amino acid sequence and the second detectably labeled antibody is reactive with said hSOD amino acid sequence.

29. The method of claim 24, wherein said second antigen that reacts with an HCV antibody from the biological sample comprises an epitope from the c22 region of the HCV polyprotein.

30. The method of claim 29, wherein the epitope from the c22 region comprises amino acids Lys_{10} to Ser_{99} of the HCV polyprotein, with a deletion of Arg47 and a substitution of Leu for Trp at position 44, numbered relative to the HCV1 polyprotein sequence, wherein said epitope is fused with a human superoxide dismutase (hSOD) amino acid sequence and the second detectably labeled antibody is reactive with said hSOD amino acid sequence.

31. The method of claim 24, wherein said multiple epitope fusion antigen comprises the amino acid sequence depicted in Figures 7A-7F.

32. A method of detecting hepatitis C virus (HCV) infection in a biological

sample, said method comprising:

(a) providing an immunoassay solid support according to claim 12;

(b) combining a biological sample with said solid support under conditions which allow HCV antigens and antibodies, when present in the biological sample, to bind to the said at least two anti-core antibodies, said NS3/4a conformational epitope, and said multiple epitope fusion antigen, respectively;

(c) adding to the solid support from step (b) under complex forming conditions (i) a first detectably labeled antibody, wherein said first detectably labeled antibody is a detectably labeled HCV anti-core antibody, wherein said labeled anti-core antibody is directed against a different HCV core epitope than the at least two anti-core antibodies bound to the solid support; (ii) an epitope from the c33c region of the HCV polyprotein fused to an hSOD amino acid sequence and an epitope from the c22 region of the HCV polyprotein fused to an hSOD amino acid sequence; and (iii) a second detectably labeled antibody, wherein said second detectably labeled antibody is reactive with said hSOD amino acid sequences;

(d) detecting complexes formed between the antibodies and antigens, if any, as an indication of HCV infection in the biological sample.

33. The method of claim 32, wherein said at least two anti-core antibodies are directed against an N-terminal region of the HCV core antigen and said detectably labeled HCV anti-core antibody is directed against a C-terminal region of the HCV core antigen.

34. The method of claim 33, wherein said at least two anti-core antibodies are directed against amino acids 10-53 of HCV, numbered relative to the HCV1 polyprotein sequence and said detectably labeled HCV anti-core antibody is directed against amino acids 120-130 of HCV, numbered relative to the HCV1 polyprotein sequence.

35. The method of claim 32, wherein the epitope from the c22 region comprises amino acids Lys_{10} to Ser_{99} of the HCV polyprotein, with a deletion of Arg47 and a

substitution of Leu for Trp at position 44, numbered relative to the HCV1 polyprotein sequence.

36. An immunodiagnostic test kit comprising the immunoassay solid support of claim 1, and instructions for conducting the immunodiagnostic test.

37. An immunodiagnostic test kit comprising the immunoassay solid support of claim 9, and instructions for conducting the immunodiagnostic test.

38. An immunodiagnostic test kit comprising the immunoassay solid support of claim 12, and instructions for conducting the immunodiagnostic test.

39. A method of producing an immunoassay solid support, comprising:

(a) providing a solid support; and

(b) binding at least one hepatitis C virus (HCV) anti-core antibody and at least one isolated HCV NS3/4a conformational epitope thereto.

40. A method of producing an immunoassay solid support, comprising:

(a) providing a solid support; and

(b) binding two hepatitis C virus (HCV) anti-core antibodies and an isolated HCV NS3/4a conformational epitope thereto.

41. The method of claim 39, further comprising binding at least one multiple epitope fusion antigen to the solid support.

42. The method of claim 40, further comprising binding at least one multiple epitope fusion antigen to the solid support.

43. A method of producing an immunoassay solid support, comprising:

(a) providing a solid support; and

(b) binding two hepatitis C virus (HCV) anti-core antibodies, an isolated HCV NS3/4a conformational epitope, and a multiple epitope fusion antigen, thereto.

44. A multiple epitope fusion antigen comprising the amino acid sequence depicted in Figures 7A-7F, or an amino acid sequence with at least 80% sequence identity thereto which reacts specifically with anti-HCV antibodies present in a biological sample from an HCV-infected individual.

45. The multiple epitope fusion antigen of claim 44, wherein said multiple epitope fusion antigen comprises the amino acid sequence depicted in Figures 7A-7F, or an amino acid sequence with at least 90% sequence identity thereto which reacts specifically with anti-HCV antibodies present in a biological sample from an HCV-infected individual.

46. The multiple epitope fusion antigen of claim 44, wherein said multiple epitope fusion antigen consists of the amino acid sequence depicted in Figures 5A-5F.

47. A polynucleotide comprising a coding sequence for the multiple epitope fusion antigen of claim 44.

48. A polynucleotide comprising a coding sequence for the multiple epitope fusion antigen of claim 45.

49. A polynucleotide comprising a coding sequence for the multiple epitope fusion antigen of claim 46.

50. A recombinant vector comprising:

(a) a polynucleotide according to claim 47;

(b) and control elements operably linked to said polynucleotide whereby the coding sequence can be transcribed and translated in a host cell.

51. A recombinant vector comprising:

(a) a polynucleotide according to claim 48;

(b) and control elements operably linked to said polynucleotide whereby the coding sequence can be transcribed and translated in a host cell.

52. A recombinant vector comprising:

(a) a polynucleotide according to claim 49;

(b) and control elements operably linked to said polynucleotide whereby the coding sequence can be transcribed and translated in a host cell.

53. A host cell transformed with the recombinant vector of claim 50.

54. A host cell transformed with the recombinant vector of claim 51.

55. A host cell transformed with the recombinant vector of claim 52.

56. A method of producing a recombinant multiple epitope fusion antigen comprising:

(a) providing a population of host cells according to claim 53; and

(b) culturing said population of cells under conditions whereby the multiple epitope fusion antigen encoded by the coding sequence present in said recombinant vector is expressed.

57. A method of producing a recombinant multiple epitope fusion antigen comprising:

(a) providing a population of host cells according to claim 54; and

(b) culturing said population of cells under conditions whereby the multiple epitope fusion antigen encoded by the coding sequence present in said recombinant vector is expressed.

58. A method of producing a recombinant multiple epitope fusion antigen comprising:

(a) providing a population of host cells according to claim 55; and

(b) culturing said population of cells under conditions whereby the multiple epitope fusion antigen encoded by the coding sequence present in said recombinant vector is expressed.